

## Cytological evaluation of canine lymphadenopathies - a review of 109 cases

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### ABSTRACT

Lymphadenopathy is a commonly encountered condition in canine patients. It is not a specific disease entity but an important clinical finding; the cause should be ascertained to attempt treatment and prognosis. Aspiration cytology is now gaining popularity as a valuable aid in diagnosing lymphadenopathies because of its simplicity, rapidity, early availability of results with minimal trauma and complication to the patients. Therefore the following study was conducted to evaluate the different cytomorphological patterns associated with various canine lymphadenopathies and the usefulness of fine needle aspiration biopsy (FNAB) in diagnosing these conditions. A total of 109 FNAB samples were collected from cases of clinical lymphadenopathies in dogs. The FNAB provided an adequate quantity and quality of samples for cytomorphological analysis. Air dried FNAB smears yielded satisfactory results with Romanowsky's stains, while wet fixed smears stained satisfactorily with Harris Haematoxylin and Eosin (H&E) and Papanicolaou ('Pap') stains. The cytological diagnosis made from 109 cases were, 52 reactive hyperplasia, 25 neutrophilic lymphadenitis, 15 eosinophilic lymphadenitis, 12 metastatic lymphadenopathies, 4 lymphomas and 1 plasmacytoma. From the results of this study it can be concluded that the FNAB technique and Romanowsky's stains were found to be the easy and rapid methods for lymph node sampling and staining respectively.

**Key words:** lymphadenopathy, fine needle aspiration cytology, cytology, reactive hyperplasia, lymphadenitis, lymphoma

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### Introduction

Lymph node enlargement, referred to as lymphadenopathy, is a frequently encountered problem in veterinary patients. Evidence suggests that cytology serves as a very useful tool in the diagnosis of two common conditions viz., cutaneous/subcutaneous masses and lymphadenopathies in veterinary patients (VILLIERS and DUNN, 1998). The same scenario exists in human medicine where aspiration cytology has become the primary diagnostic

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procedure for assessment of lymphadenopathy in human immunodeficiency virus patients (VANSIRI et al., 2008). Moreover cytological techniques have become an integral part of diagnosis in clinical cases of malignancy (ROSZEL, 1981). Being a simple and rapid procedure, the technique employs few resources to obtain the representative cells from most lesions. Although it is not possible to gain information on the tissue architecture, the cellular components, neoplastic and non-neoplastic cells could be interpreted clearly with the help of cytology (MORRIS and DOBSON, 1992).

The most preferred way of obtaining a diagnostic cytological sample from a peripheral and/or internal lymph node is by fine needle aspiration biopsy (FNAB) or non-aspiration fine needle biopsy (COWELL et al., 2003). Furthermore, the interpretations of the FNAB technique were 97% accurate when compared with the histological findings in human cases of lymphadenopathies (STEWART et al., 1998). FNAB of lymph nodes offer a quick assessment of the underlying process as it helps to differentiate reactive hyperplasia, inflammation and neoplasia, whether it originates from cells resident in or circulating through the node or from metastatic lymphatic spread from malignant tumours. TESKE and HEERDE (1996) stated that the cytological examination of FNAB specimens has been generally accepted as a reliable technique for diagnosing malignant lymphoma in dogs. This was further substantiated by BULEY (1998) who reported that the sensitivity and specificity of FNAB of lymph nodes for metastatic malignancy was 98%.

Air dried FNAB smears yield cells with optimal cytoplasmic and nuclear details (MORRIS and DOBSON, 1992). The nuclear and nucleolar details were sufficient to differentiate neoplasia and inflammation and for cytological evidence of malignant potential with Romanowsky's stain (MEINKOTH and COWELL, 2002). When air dried cells were stained with Haematoxylin and Eosin (H&E) there was marked loss of nuclear details when compared with the cells that were rehydrated or immediately fixed with 95% ethanol (LUMSDEN and BAKER, 2000). This is supported by the fact that rehydration and fast Papanicolaou's or H&E staining of air dried aspirates or impression smears yielded better nuclear and nucleolar details and has potential value in the characterization of lymphocytic nuclei (TAYLOR and BAKER, 2000). The increased use of Papanicolaou staining for cytological diagnosis of malignancy was brought to light by George Papanicolaou. However, it was later found that Papanicolaou staining was inadequate for lymphoid evaluation (MAGNOL et al., 1994). With this collective knowledge, the present study was contemplated to evaluate the cytological features in canine lymphadenopathies with the help of FNAB.

### **Materials and methods**

*Sample collection.* The FNAB samples of lymph nodes were collected from dogs with clinical lymphadenopathies, attending clinics at Madras Veterinary College Teaching Hospital, Chennai, India.

*Cytology techniques and staining.* FNAB samples were collected according to the methods of MILLS (1984) and COWELL et al. (2003). Smears were either wet fixed by using 95% ethanol (SACHDEVA and KLINE, 1981) for 20 minutes or air dried rapidly, to be subsequently stained with Romanowsky's stains. The methods described by MAGNOL et al. (1994) were used for staining the smears with May-Grünwald (MG), May-Grünwald-Giemsa (MGG), Wright's and Wright's-Giemsa (WRG) stains. Leishman-Giemsa (LG) staining was done by flooding the smear with a LG stain for a minute and diluting it with double the quantity of distilled water. The smear was left undisturbed for 20 minutes and then examined. Wet fixed smears were stained with Harris H&E staining as per the techniques of BANCROFT and STEVENS (1996). The methodology for Papanicolaou staining was adapted from SACHDEVA and KLINE (1981).

### Results

The cellular details described here were based on the Romanowsky's type stained smear, except where mentioned. The nuclei stained purplish and the cytoplasm pale basophilic. Mast cell granules stained purplish. Air dried FNAB smears yielded good cytoplasmic and nuclear details with Romanowsky's type stains. Thick smears obtained from metastatic lesions with dense clusters and multiple layers of neoplastic cells and lymphomas, wet fixed in 95% ethanol, showed good nuclear and nucleolar details when stained by the 'Pap' and H&E methods.

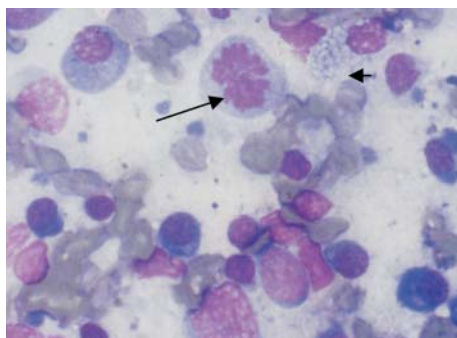


Fig. 1. Lymph node smear. Reactive hyperplasia showing mitotic figure (arrow), plasma cells and a mott cell (arrow head) with Russell bodies (WRG;  $\times 1000$ )

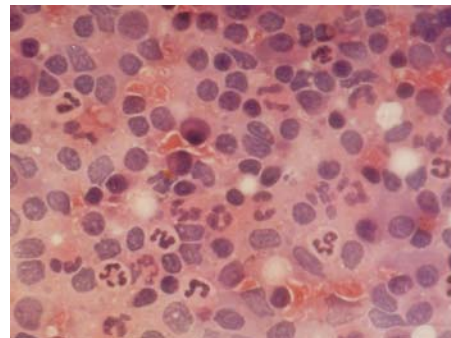


Fig. 2. Neutrophilic lymphadenitis showing intact and degenerate neutrophils (H&E;  $\times 800$ )

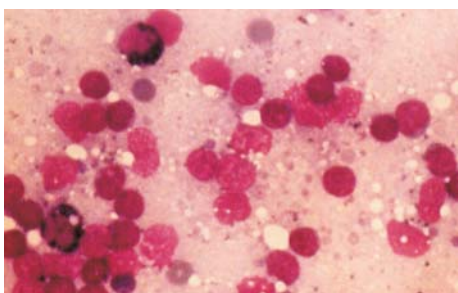


Fig. 3. Eosinophilic lymphadenitis (LG; ×800)

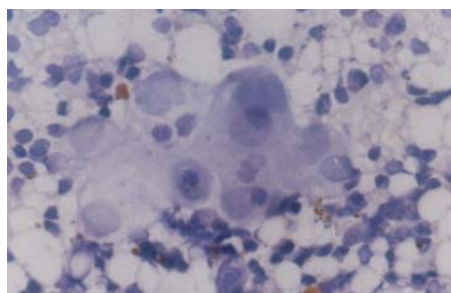


Fig. 4. Lymph node. Metastatic squamous cell carcinoma with clusters of round to polyhedral cells, hyperchromatic nuclei and basophilic nucleoli. ('Pap'; ×800).

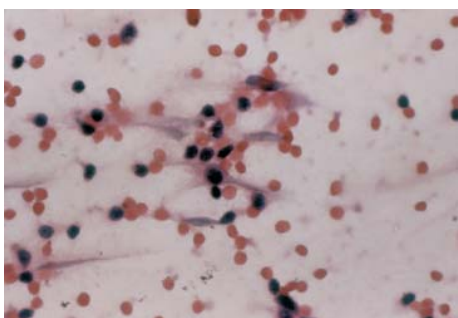


Fig. 5. Lymph node. Spindle cells with deeply eosinophilic cytoplasm and round to oval basophilic nuclei in metastatic sarcoma (H&E; ×800)

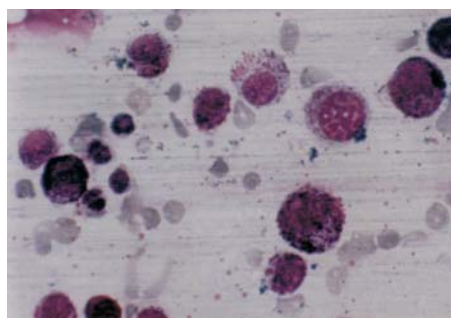


Fig. 6. Lymph node. Mast cells with red purple cytoplasmic granules as seen in metastatic mast cell tumour (WRG; ×1000).

Table 1. Mean ± SE percentage and range of cells in FNAB cytology smear

Cytological diagnosis	Small lymphocytes	Large lymphocytes	Lymphoblasts	Plasma cells	Neutrophils	Eosinophils
Reactive hyperplasia	59.70 ± 2.46	10.19 ± 0.14	15.02 ± 1.51	13.46 ± 1.82	0.56 ± 0.15	0.13 ± 0.07
Neutrophilic lymphadenitis	61.88 ± 2.29	8.52 ± 1.32	9.48 ± 1.54	7.72 ± 1.50	11.76 ± 1.63	1.00 ± 0.47
Eosinophilic lymphadenitis	44.87 ± 3.98	10.40 ± 2.54	18.53 ± 1.61	14.40 ± 3.48	2.80 ± 0.81	9.60 ± 1.91
Metastatic lymphadenopathy	54.58 ± 5.08	5.75 ± 0.63	15.08 ± 3.36	17.73 ± 3.25	1.17 ± 0.56	-

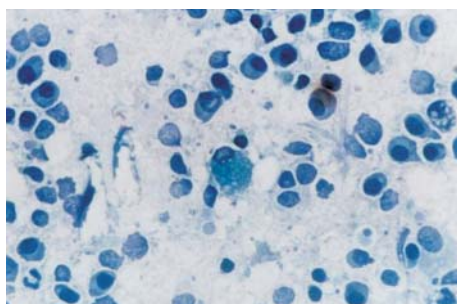


Fig. 7. Lymph node. Plasmacytoma showing round to polyhedral cells, prominent anisocytosis and anisokaryosis. Mott cell with Russell bodies ('Pap'; ×800)

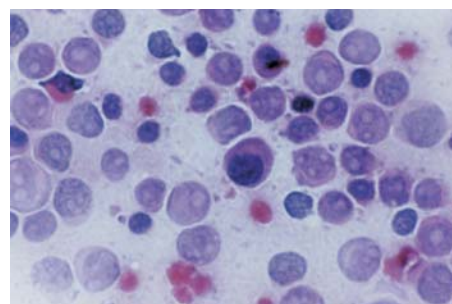


Fig. 8. Lymphoma. Lymphoblasts with macronuclei and multiple nucleoli and moderate amount of eosinophilic cytoplasm (H&E; ×1000)

The mean ± S.E percentage of cells observed in lymph node smears are shown in Table 1. Out of 109 cases, 52 were diagnosed as reactive hyperplasia. In a few cases, the lymphoblasts with mitosis were associated with immunoblast, showing hyperbasophilic cytoplasm. There was a distinct increase in the plasma cells in different stages of differentiation (appearance of clear Golgi zone, cytoplasmic vacuoles) with the moderate to occasional presence of mott cells with Russell bodies (Fig. 1). Mild to moderate amount of tingible body macrophages (TBM) and lymphoglandular bodies were present.

Twenty-five cases were diagnosed as neutrophilic lymphadenitis. There was an absolute increase in neutrophils in only eight cases (Fig. 2). The remaining cases showed a neutrophilic response accompanied by hyperplastic changes i.e. increased amount of lymphoblasts and plasma cells. Eosinophilic lymphadenitis was diagnosed in 15 cases (Fig. 3).

Table 2. Tumor, node and metastasis staging of lymphomas

Case details	Clinical signs	Stage	Cells on cytology smear
Case I	Generalised lymph node enlargement, hepatosplenomegaly, involvement of bone marrow with systemic signs	V b	SL 15%, ML 5%, LB 80%
Case II	Generalised lymph node enlargement, hepatosplenomegaly with systemic signs	IV b	SL 18%, LB 82%
Case III	Generalised lymph node enlargement with systemic signs	III b	SL 9%, ML 1%, LB 88%, N 2%
Case IV	Peripheral lymph node enlargement with systemic signs	II b	SL 15%, ML 5%, LB 80%

SL-Small lymphocytes; LB-Lymphoblasts; ML-Medium lymphocytes; N-Neutrophils

Table 3. Metastatic lymphadenopathies

S. No.	Primary tumour	Lymph node sample	Metastasis as seen on cytology
I Carcinomas			
1.	Mammary adenocarcinoma	Inguinal	+
2.	Mammary adenocarcinoma	Inguinal	+
3.	Mammary adenocarcinoma	Inguinal	-
4.	Mammary squamous cell carcinoma	Inguinal	+
5.	Ethmoidal squamous cell carcinoma	Submandibular	+
6.	Ethmoidal adenocarcinoma	Submandibular	+
7.	Ethmoidal anaplastic cell carcinoma	Submandibular	+
8.	Sweat gland adenocarcinoma	Submandibular	+
9.	Epulis squamous cell carcinoma	Submandibular	-
II Sarcomas			
10.	Epulis - Fibrosarcoma	Submandibular	+
11.	Synovial sarcoma	Popliteal	-
III Round cell tumour			
12.	Mast cell tumour	Inguinal	+

Out of 109 cases, nine were classified as metastatic lymphadenopathies with the evidence of metastasis, involving seven carcinomas, one sarcoma and one mast cell tumour. Three cases were diagnosed as tumour associated lymphadenopathy, without any metastatic lesions (Table 3). The cells of the metastatic carcinoma appeared as large round to polyhedral clusters with round to oval vesicular nuclei. Nuclei were hyperchromatic and some of the cells had multiple basophilic nucleoli. The cytoplasm was intensely basophilic and some showed vacuolation (Fig. 4). Sarcoma cells appeared to be spindle shaped with deep eosinophilic cytoplasm with tails and deep basophilic round to oval nucleus with indistinct nucleolus (Fig. 5). The cells of the mast cell tumour were round containing small reddish-purple granules in the cytoplasm and had round nuclei with indistinct nucleoli (Fig. 6).

One of the 109 cases was identified as plasmacytoma. In a 'Pap' stained smear, the cells were round to polyhedral with discrete margins. Anisocytosis and anisokaryosis were prominent. Mott cells with Russell bodies were also seen (Fig. 7).

Four cases were recorded as lymphomas from the 109 cases. Table 2 shows the World Health Organization TNM (Tumour, Node and Metastasis) staging of lymphomas and the percentage of cells as seen on cytology smears. Most of the cells were lymphoblasts (80%) having deep blue granular scanty cytoplasm with multiple nucleoli. The lymphoblasts had a moderate to a thin rim of pale eosinophilic cytoplasm and a pale blue round to cleaved

nuclei with single to multiple large nucleoli in most of the cells in H&E stained smears (Fig. 8). Anisocytosis, anisokaryosis, macronuclei and multiple nucleoli were observed in 'Pap' stained smears.

### Discussion

Air dried smears stained with Romanowsky's stains allowed satisfactory interpretation of cytological biopsies. Wright's, May-Grünwald and Leishman stains when combined with Giemsa yielded better nuclear and cytoplasmic details. However, Romanowsky's stain was inferior to 'Pap' stain in evaluating irregularities in chromatin and nucleoli. These results were comparable with the observations of MAGNOL et al. (1994). Nuclear details were better discernible in H&E and 'Pap' stains when compared to the Romanowsky's stains. These observations were in accordance with LUMSDEN and BAKER (2000). However, the 'Pap' stain was inadequate for lymphoid evaluation as reported by MAGNOL et al. (1994).

Reactive hyperplasia showed a 27 and 7 fold increase in the mean percentage of plasma cells and lymphoblasts, respectively. Correspondingly there was a decrease in the number of small lymphocytes. These findings concurred with those of DUNCAN (1993). A few mast cells, mitotic figures, and mott cells with Russell bodies accompanied the reactive hyperplasia as reported by THRALL (2000) and COWELL et al. (2003).

A 10 fold increase in the neutrophils and a 9 fold increase in the eosinophils were observed in cases of neutrophilic and eosinophilic lymphadenitis, respectively. Only 32% of the cases showed an absolute neutrophilic lymphadenitis, where as all the eosinophilic lymphadenitis revealed a mixed reaction with an increase in neutrophils, lymphoblasts and plasma cells. Comparatively the percentage of lymphoblasts and plasma cells was higher in eosinophilic lymphadenitis and the mean percentage of small lymphocytes was lower than any other lymphadenopathies. COWELL et al. (2003) stated that an increased number of plasma cells were usually present with lymphadenitis of any cause as was observed in the study.

The percentage of metastasis to regional lymph nodes observed in this study was high when compared to the report of LAGENBACH et al. (2001), i.e. 43.75% for carcinomas and 12.50% for sarcomas. The higher percentage of detection might be due to the low number of cases observed in this study. However, FNAB was highly sensitive for detecting metastatic lesions in the lymph nodes.

Moderately differentiated mast cell tumours had higher potential for metastasis to regional lymph nodes regardless of the lesion. This should not be mistaken for residual or reactive mast cells which are occasionally observed. In dogs, cutaneous mast cell tumours in the inguinal regions frequently metastasized to the abdominal lymph nodes

(ALLENMAN and BAIN, 2000). In this study, an inguinal lymph node with mast cell tumour metastasis was occupied by neoplastic cells with cytoplasmic reddish purple granules on Romanowsky's stained smears. The cytological criteria of anisocytosis and anisokaryosis observed in the metastasized carcinomas and sarcomas were in close agreement with previous reports (MILLS, 1984; DUNCAN, 1993; MAGNOL et al., 1994; COWELL et al., 2003). Thus the FNAB technique was found to be superior to impression and scraping methods and histopathology in detecting metastatic neoplasm in the lymph nodes.

Lymphoma revealed a mean percentage of 82.2% of lymphoblasts. These results were comparable to an earlier description of lymphoblastic lymphoma by BURKHARD and MEYER (1996). BARRET (1978) and CANIATTI et al. (1996) reported the cell population as 50-80% in the lymphoblastic lymphoma. All the four cases encountered were lymphoblastic type and had high mitotic index in one, medium in another and low in two other dogs. Cell fragility, bare nuclei, and lymphoglandular bodies were numerous in lymphoma than in hyperplasia. Most of the cells (>80%) populating the node were blasts. These observations agreed with those of earlier workers (CANIATTI et al., 1996; THRALL, 2000; NESBIT et al., 2002). Morphologically, a neoplastic lymphoblast appeared almost like a non-neoplastic lymphoblast. Therefore it was the percentage of lymphoblasts and not the malignant criteria that allowed for recognition of lymphoma (COWELL et al., 2003)

The present study showed that the FNAB of lymph nodes yielded high cellularity and adequate quality and quantity of samples for satisfactory cytological interpretation. Romanowsky's staining on air dried smears was sufficient in differential diagnosis of lymphadenopathies. 'Pap' and H&E staining on 95% ethanol fixed cytological smears may be preferred for diagnosing lymphomas and metastatic neoplasia. This paves the way for routine employment of cytological technique in the quick diagnosis of lymphadenopathies in canine practice.

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M. Thangapandiyan and C. Balachandran: Cytological diagnosis of canine lymphadenopathies

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**THANGAPANDIYAN, M., C. BALACHANDRAN: Citološka prosudba limfadenopatija u pasa - pregled 109 slučajeva. Vet. arhiv 80, 499-508, 2010.**

**SAŽETAK**

Limfadenopatija se često javlja u pasa i nije zasebna bolest nego važan klinički nalaz čiji se uzrok mora ustanoviti da bi se moglo poduzeti liječenje i dati prognoza. Citologija danas dobiva na važnosti kao dragocjena pomoć pri dijagnosticiranju limfadenopatija zbog svoje jednostavnosti, brzine te brzog dobivanja rezultata s minimalnom traumom i komplikacijama za pacijente. Ovo istraživanje poduzeto je s ciljem da se procijeni vrijednost različitih citomorfoloških uzoraka povezanih s različitim limfadenopatijama u pasa te upotrebljivost aspiracijske biopsije radi dijagnosticiranja limfadenopatija. Ukupno je biopsijom bilo uzeto 109 uzoraka tkiva od pasa s kliničkim limfadenopatijama. Aspiracijska biopsija pruža mogućnost uzimanja uzoraka za citomorfološku pretragu odgovarajuće veličine i kakvoće. Na zraku osušeni razmasci uzetoga tkiva, obojeni po Romanovskom, daju zadovoljavajuće rezultate, dok su se vlažno fiksirani razmasci tkiva zadovoljavajuće obojili hematoksilin-eozinom po Harrisu i bojenjem po Papanicolaouu. Pretragom 109 uzoraka tkiva pacijenata postavljena je citološka dijagnoza reaktivna hiperplazija u 52 pacijenta, neutrofilni limfadenitis u 25, eozinofilni limfadenitis u 15, metastatske limfadenopatije u 12, limfom u četiri i plazmocitom u jednog pacijenta. Može se zaključiti da su aspiracijska biopsija i bojenje po Romanovskom lako i brzo izvedive metode za uzimanje uzoraka limfnih čvorova i bojenje uzetoga tkiva.

**Ključne riječi:** limfadenopatija, aspiracija tkiva, citologija, reaktivna hiperplazija, limfadenitis, limfom

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